

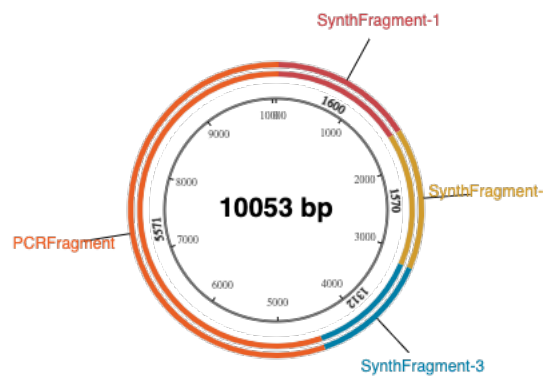
## New\_Assembly

Created: 12/29/2021, 6:52:17 PM

Saved: not saved

### Component Fragments

Name	Length	Produced by	5' End	3' End
SynthFragment-1	1600	Synthetic	---	---
SynthFragment-2	1600	Synthetic	---	---
SynthFragment-3	1342	Synthetic	---	---
PCRFragment	5630	PCR	Fwd Primer (auto)	Rev Primer (auto)



### Notes

- For assemblies of 4 or more fragments, we recommend using overlaps of at least 25 bp when using NEBuilder.
- A 60 minute reaction is recommended for the assembly of more than 3 fragments.

### Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
PCRFragment_fwd	GGCAATCAGGCAGTAATG	18	50	50	60.7	59.8
PCRFragment_rev	TAACTGTCAGACCAAGTTTAC	21	38	38	58.8	59.8

### Build Settings

Property	Value
Product/Kit	#E5520 NEBuilder HiFi DNA Assembly Cloning Kit
Minimum Overlap	20 nt
Minimum Overlap Tm	48 °C
Circularize	Yes
PCR Polymerase/Kit	Q5 High-Fidelity DNA Polymerase
PCR Primer Conc.	500 nM
Min. Primer Length	18 nt

## Assembled Sequence

```
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#ACCESSION    .
#VERSION      .
#KEYWORDS     NEBuilder
#SOURCE       synthetic DNA construct
# ORGANISM    synthetic DNA construct
#REFERENCE    1 (bases 1 to 10053)
# AUTHORS     .
# TITLE       NEBuilder-generated Construct
# JOURNAL      Exported 29-DEC-2021 from NEBuilder https://nebuilder.neb.com
#COMMENT      NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
#COMMENT      PCRFragment_fwd: GGCAATCAGGCAGTAATG
#COMMENT      PCRFragment_fwd 3'Tm: 60.7 3'Ta: 59.8
#COMMENT      PCRFragment_rev: TAACTGTCAGACCAAGTTTAC
#COMMENT      PCRFragment_rev 3'Tm: 58.8 3'Ta: 59.8
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